PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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Christine Todd Whitman, Administrator
US Environmental Protection Agency
Ariel Rios Building (1101A)
1200 Pennsylvania Ave. NW
Washington, DC 20460

Re: Comments on ACC's Alkenyl Succinic Anhydride Category

Dear Administrator Whitman:

The following are comments on the American Chemistry Council's revised test plan for the Alkenyl Succinic Anhydride Category. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal and environmental protection organizations have a combined membership of more than ten million Americans.

We are disappointed to once again find that a category of likely non-toxic chemicals with minimal or no water solubility, high viscosities, high molecular weights, and little environmental mobility is being proposed for additional acute fish toxicity testing and mammalian testing for several SIDS endpoints as listed below:

- acute fish toxicity (40-120 fish if OECD No. 203 is followed)
- *in vitro* chromosomal aberration assay (we propose human lymphocytes instead of Chinese hamster ovary or lung cells or rat or mouse bone marrow cells)
- repeat dose (28-day) toxicity (40-65 rats if OECD No. 407 is followed)
- one-generation reproduction toxicity (1,300 rats if OECD No. 415 is followed)

The test plan as currently presented will kill up to 1,485 animals.

Category Issues

We appreciate the fact that the ACC combined the two anhydride compounds in the category with the diacid compounds, since any exposure would result in the immediate conversion of the anhydrides to the diacid. This categorization is logical and straightforward. However, the compounds in this category should be further evaluated in light of the information gained from test plans involving other similar compounds such as Dupont's Dicarboxylic Acid Category, where high molecular weight dicarboxylic acids were found to have low toxicities. The compounds in this category are likely to have even lower toxicities, as the molecular weights are heavier and all the chemical/physical properties of these chemicals point to very low mobilities. The vast weight of evidence for compounds with long branched alkane/alkenes chains with a polar end to them shows that they tend to have very low toxicities . See also, for example, the ACC's alkaryl sulfonate category and our comments on that testing plan. Furthermore, the exposure to workers and consumers of chemicals in this category will typically be as trace or

minor constituents in lubricating oils or their additives, so that exposure will be at dramatically lower levels than the levels of testing that have already been conducted.

Testing Issues

In consideration of all these facts, it is senseless to kill more animals merely to check the boxes in the SIDS test list. In the cover letter to the test plan, ACC claims that "careful consideration was given to the number of animals that would be required for tests included in the proposed plan and conditions to which the animals might be exposed. In consideration of the concerns of some non-governmental organizations about animal welfare, **the use of animals in this proposed test plan has been minimized**" (emphasis added).

While it is nice that the ACC mentions animal welfare issues, the fact is that the test plan fails miserably to take humane issues into consideration, as the following examples amply illustrate:

1. The water solubility is stated to be "sparingly low to insoluble" and the $K_{o/w}$ was calculated to be 4.8. Still the ACC, despite their stated intentions concerning minimizing animal use, proposes to kill fish in aquatic toxicity testing. Even the EPA has clearly stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of acute fish and *Daphnia* tests (EPA *Federal Register,* December 2000, p. 81695). The log $K_{o/w}$ value of CAS 68227-46-3 has been calculated to be 4.38 (robust summaries, p. 1), and there is no plan to test this value experimentally (test plan, p. 5). Per the EPA's instructions, the fish test should therefore not be carried out. The fact that the solubility is apparently so low that the planned tests have to use an aqueous suspension rather than solution (p. 4) further supports the inappropriateness of this test.

2. The ACC does not state the cell type to be used in the *in vitro* chromosomal aberration assay. Although this is considered to be an *in vitro* assay, it is not non-animal, as various animal tissues are used (Chinese hamster ovary or lung cells, human or rat lymphocytes, or human, rat or mouse bone marrow). We recommend that human lymphocytes be used to eliminate the need to kill animals to test this endpoint.

3. Most importantly, the ACC is proposing to conduct two separate tests to assess sub-chronic (40 – 65 rats if OECD No. 407 is followed) and reproductive toxicity in a 1-generation reproduction study (1300 animals if OECD No. 415 is followed). These two tests alone will kill up to 1365 animals. While the ACC discusses the OECD combined protocol (No. 422) on page 19 of the test plan, for reasons unknown and in spite of its stated intentions to minimize animal use, **the ACC inexplicably proposes to kill twice as many animals to meet this SIDS endpoint**. In addition, please see the information on the rodent embryonic stem cell test listed in the Appendix to these comments.

4. Finally, the expected low toxicity of these compounds only further obviates the perceived need to kill more animals merely to document an already anticipated

outcome. These high molecular weight molecules with long branched alkane/alkene side chains with a polar end are unlikely to be toxic. If the ACC wishes to carry out the tests indicated in the test plan, there is a range of *in vitro* and *in silico* alternatives to fish and acute and developmental mammalian toxicity tests, as detailed in the Appendix to these comments. The ACC, as the main industry proponent of the HPV program, should be actively pursuing with the EPA the use of these *in vitro* tests.

In summary, the ACC test plan provides lip service to its "concerns about minimizing animals" while at the same time proposing clearly unnecessary tests that will kill an extremely large number of animals. The ACC has failed to use thoughtful toxicology or even protocols that are accepted under SIDS to minimize animal use. We urge the ACC to reconsider its proposal and to take the issue of animal suffering and death in the HPV program – and in this test plan in particular – more seriously. Before proposing to kill any more animals, the ACC should consider the information gleaned from such examples as the dicarboxylic acid category regarding high molecular weights and low toxicities, reconsider the need to kill fish with a non-water soluble compound, and use thoughtful toxicology to circumvent additional testing on animals for the substances in this category. The EPA should reject this plan and provide guidance to the ACC on the principles of its October 1999 letter addressing animal use.

Thank you for your attention to these comments. The organizations listed in the introductory paragraph of these comments request that the ACC contact us directly to discuss these concerns.

Sincerely,

Jessica Sandler
Federal Agency Liaison

cc: Steven Russell and Larry Rampy, ACC

Appendix: *In vitro* and *in silico* test methods

1. In silico *fish test substitute*. Quantitative structure activity relationship (QSAR) programs provide *in silico* methods for estimating toxicity to fish and other aquatic organisms. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

2. In vitro *fish test substitutes*:

   (i) TETRATOX is an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997). With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997), and the extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After more than one year, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for the ACC to work with the EPA on incorporating a non-animal test into the HPV program.

   (ii) The test protocol and performance parameters of the recently validated *Dar*T test are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, it uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

3. *Mammalian developmental toxicity test substitute. In vivo* developmental and reproductive toxicity tests have not been validated for humans. However, an *in vitro* embryotoxicity test

method, the rodent embryonic stem cell test, has recently been validated by the European Centre for the Validation of Alternative Methods, and the Centre's Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). If a positive result is found in the embryonic stem cell test, 68227-46-3 should be treated as a development toxicant/teratogen, and no further testing should be carried out within the HPV screening-level program. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV Program, with correspondence dating back more than six months, we have received no reply. We urge the ACC to correspond directly with the EPA on the incorporation of this validated non-animal test.